PATENT
Docket No.: PIT-010

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Michael B. CHANCELLOR et al.		
Serial No.	:	09/302,896	Art Unit:	1636
Filed	:	April 30, 1999	Examiner:	Sumesh Kaushal
For	:	**Muscle-Derived Cells (MDCs) for Treating Muscle- or Bone-Related Injury or Dysfunction (As Amended)**		

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Date: December 8, 2003

Signature: Leslie Serunian
Leslie Serunian, Reg. No. 35,353

Declaration of Michael B. Chancellor, M.D. under 37 C.F.R. §1.132

I, Michael B. Chancellor, M.D., hereby declare and state that:

1. I am an inventor of subject matter described and claimed in the above-identified patent application (referred to as the "Chancellor application" herein).

2. I am a member of the faculty in the Department of Urology at the University of Pittsburgh School of Medicine, Pittsburgh, PA. As part of my responsibilities as a physician and faculty member, I perform clinical studies and direct and carry out research, primarily in the field of urological injury, damage and dysfunction. The research in my laboratory focuses on developing and testing new treatments and therapies for diseases and disorders in the field of urology, with an emphasis on stress urinary incontinence (SUI). In collaboration with my colleagues, I and my laboratory

researchers perform research in the areas of muscle-derived cell (MDC)-based treatments for a variety of different urological disorders using animal (e.g., rat and mouse) model systems. In particular, animal models of SUI are studied to discover new treatments and therapies for SUI and related disorders of the genitourinary system, for eventual application in human patients.

3. I have reviewed the Chancellor application and claims, and have read and understood the complete contents of the final office action mailed from the U.S. Patent and Trademark Office on July 9, 2003, including the Examiner's comments and rejections of the claims at pages 2-7 of the Detailed Action.

4. In the office action, the Examiner has rejected claims 119-195 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art ... to make and/or use the invention.

5. Based on the teachings of the Chancellor application, members of my laboratory, under my direction, have conducted experiments using MDCs isolated from skeletal muscle (i.e., gastrocnemius muscle) by the method taught in the application, in a rat animal model of incontinence, i.e., intrinsic sphincteric deficiency, a form of stress urinary incontinence (SUI). Our results show that MDCs are present at several weeks following injection into the urethra wall, as determined by LacZ staining, as also taught in the application. In addition, our results clearly demonstrate that urethral injections of MDCs improve continence in the rat model of incontinence, as determined by an increase in leak point pressure (LPP), which is an art-recognized parameter for

assessing treatments of urinary incontinence, including SUI, as mentioned in the Chancellor application at page 8.

6. A first set of experiments related to the treatment of urinary incontinence using MDCs as obtained and described in the Chancellor application and as referred to in ¶5 above is presented in Appendix 1, attached at Tab 1. These experiments were conducted to study and evaluate the therapeutic effects of periurethral MDC injection in a rat model of stress urinary incontinence in which the urethra was cauterized to simulate urethral injury and dysfunction. Appendix 1 contains the experimental methods, results and figures related to this first set of experiments.

7. A second set of experiments related to the treatment of urinary incontinence using MDCs as obtained and described in the Chancellor application and as referred to in ¶5 above is presented in Appendix 2, attached at Tab 2. Appendix 2 contains the experimental methods, results and figures related to this second set of experiments.

8. Based upon my experience and knowledge in the area of treatments for incontinence, it is widely recognized and accepted among those with knowledge in this field that bulking or augmentation of the sphincter is an appropriate and conventional means of treating a genitourinary tissue dysfunction, such as SUI. Indeed a number of non-cell bulking agents, e.g., collagen, microplastique, fat, blood, silicone, microspheres, self-detachable balloon systems and microcarbon particles, have been proposed and used to successfully treat SUI. (*See, e.g.,* FDA Guidelines, attached at Tab 3, and discussed in ¶9 of my declaration). Bulking agents work by increasing the outflow resistance of urine from the bladder into the urethra, as quantified by leak point pressure (LPP) and not by acting upon afferent nerve reflexes directly. In addition, LPP

is accepted among clinicians as the objective outcome parameter for determining efficacy of SUI treatments and assessing improvement in continence. In 1995, the FDA relied on LPP measurements in deciding to approve collagen as a periurethral bulking agent. On this basis, it is my belief that MDCs would be evaluated by the same criteria as the above-mentioned materials for their efficacy in serving as bulking agents to repair or ameliorate genitourinary tissue that is injured, damaged, or dysfunctional.

9. Based on my knowledge in the field and further to ¶7 above, I note that the FDA has published guidance for preclinical and clinical investigations of urethral bulking agents used in the treatment of urinary incontinence, i.e., the November 29, 1995, "Draft Guidance for Preclinical and Clinical Investigations of Urethral Bulking Agents used in the Treatment of Urinary Incontinence" of the Urology and Lithotripsy Devices Branch, Division of Reproductive, Abdominal, Ear, Nose and Throat and Radiological Devices, Office of Device Evaluation, Center for Devices and Radiological Health. A copy of this guide is attached hereto at Tab 3. A primary objective outcome parameter for such treatments is LPP (valsalva leak point pressure), as set forth in the FDA guidelines with respect to pre- and post-treatment clinical evaluations (Section VI.D.4. and Section VI.E.6.). As stated, in part, in Section D (Pre-treatment Evaluation), "[t]he patients who are found to have UI [Urinary Incontinence], likely due to ISD [Intrinsic Sphincter Deficiency] ... should undergo the following evaluations to confirm the diagnosis of UI due to ISD ... .

> valsalva leak point pressure (LPP) - (LPP is the intravesical pressure at which urine leaks around the catheter through the urethra during urodynamic evaluation. ..... . The clinical protocol should include a description of the procedure used for LPP determinations); ....

Thus, the FDA recognizes LPP as an important physiological parameter that is used to establish the condition of urinary incontinence and is to be considered in evaluating potential treatments for this condition.

10. It is known in the field that urethral afferent nerve activity does not cause stress urinary incontinence. Instead, stress incontinence can induce or increase urethral afferent activity. The activation of the urethral afferent nerve reflexes, caused by a weak sphincter muscle, can result in overactive bladder and urge incontinence. *See, e.g.,* S.Y. Jung et al., 1999, *J. Virol.*, 162:204-212, a copy of which has been previously provided to the Examiner.

11. The invention described and claimed in the Chancellor application provides a treatment for patients afflicted with genitourinary tract injury or dysfunction associated with urinary incontinence, as well as for patients having mixed incontinence, in which a SUI component of the affliction can be particularly treated. Based on my knowledge and experience in this field, cryo-induced injury to the urethra in rodents, such as rats and mice, is an art-recognized and accepted animal model system in which to test and evaluate treatments and therapies for genitourinary tract disorders and dysfunctions, such as SUI, for patients having such disorders and dysfunctions. Also, based on my knowledge and experience, I attest that sphincter muscle bulking or augmentation, for example, by injection of MDCs, successfully treats urinary incontinence, or SUI, in the absence of direct treatment of afferent nerve reflexes.

12. With respect to the above ¶10, and the disclosure of S.Y. Jung et al., the Examiner may be misinterpreting the cause-effect relationship between SUI and urge incontinence. While urge incontinence is facilitated by stress urinary incontinence, urge

incontinence is not a cause of stress urinary incontinence. In addition, SUI can be successfully treated without direct modulation of the afferent nerve reflexes that are associated with urge incontinence. Stress and urge incontinence can be considered disparate dysfunctions, each of which may be treated separately without detriment to the efficacy of the treatment for one or the other of the dysfunctions.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 12/08/03

By: _____
Michael B. Chancellor, M.D.

Express Mail Label No.: EV 324102475 US

Appendix 1

Materials and methods for the first set of experiments as described in ¶6 of the Chancellor Declaration

Animals and study design. Normal female Sprague-Dawley (SD) rats (Hilltop Lab Animals, Inc., Scottdale, PA), 6 weeks old, weighing 250 to 300 grams, were used in experiments to study the therapeutic efficacy of intraurethral injections of muscle derived cells (MDCs) obtained by the muscle cell plating/culturing method in a rat model of intrinsic sphincteric deficiency. In one animal group, the urethra was cauterized, followed a week later by the injection of MDCs into the urethral tissue. These rats were divided into 3 groups, which were evaluated 2 weeks (n=8), 4 weeks (n=5), and 6 weeks (n=3) after MDC injection. In another group, 9 rats underwent cauterization, followed by injection with Hank's Balanced Salt Solution (HBSS) one week later. This group served as a control for the MDC injection. The 9 rats of the control group were divided into 3 groups of 3 rats each, which were evaluated 2, 4 and 6 weeks after injection of HBSS. As a sham control, 9 normal rats underwent a sham operation during which the urethra was exposed but not cauterized.

Electrocauterization. Each rat was anesthetized with halothane (2%) and placed in the supine position with the lower legs abducted. The bladder and urethra were exposed through a lower midline abdominal incision. Tissues 1 cm lateral to the mid-urethra were cauterized on both sides to produce sphincteric injury. A fine tip, high temperature cautery (Aaron Medical, St. Petersburg, FL) was used to perform the cauterization. Each side was cauterized for 30 seconds.

Purification and labeling of MDCs. MDCs were harvested from the gastrocnemius muscles of normal adult female SD rats. The muscle was minced into a coarse slurry using successively smaller needles. Cells were enzymatically dissociated

by adding 0.2% collagenase type XI for 1 hour at 37°C, 240 units of grade II dispase for 45 minutes, and 0.1% trypsin for 30 minutes. The plating and culturing technique described in the Chancellor application was used to obtain MDCs for the MDC injections in the experiments as described. Using this technique, early preplate cells and fibroblasts adhered to the first flask. The early preplate cells were discarded because they did not proliferate well and they survived poorly after transplantation. As shown in **Figure 1a** and **Figure 1b** attached hereto, 65% of the cells from the last plating on day 6 following initiation of the culture stained positive for desmin, a myogenic marker; these comprise the MDCs used in the experiments described here. Also, when these rat MDCs were injected into the gastrocnemius muscle of an *mdx* mouse, an animal model of Duchenne muscular dystrophy with dystrophin-deficient muscle, they produced a large amount of dystrophin (**Figure 1c**). The MDCs obtained from the plating and culturing technique were plated in T75 flasks, rinsed in HBSS, and incubated for 28 hours at 37°C with MFG-NB, a retroviral vector containing a modified LacZ gene. The titer of the viral stock had a multiplicity of infection (MOI) of 500.

MDC injections. One week after electrocauterization, a 3/10 cc insulin syringe was used to inject 10 μl of MDCs obtained as described above and suspended in HBSS, (approximately 7.5×10^5 cells) into each lateral wall of the mid-urethra. A total of 20 μl (i.e., 1.5×10^6 cells) of MDC were injected into each urethra. Each control rat received injections of HBSS only.

Cystometry. At 2, 4, or 6 weeks after either MDC or HBSS injection, the rats were anesthetized with urethane (1.2 g/kg.). Thereafter, a transvesical catheter with a fire-flared tip (PE-90 tubing) was inserted into the dome of the bladder. The intravesical catheter was connected via a 3-way stopcock to a pressure transducer and a syringe pump for recording intravesical pressure and infusing saline into the bladder, respectively. Saline was infused at a rate of 0.04 ml/min to elicit repetitive bladder

contractions. The saline infusion was continued for at least 3 hours before leak point pressure (LPP) testing. Data were collected with the Windaq software package (Dataq Instruments Co., Akron, OH).

Leak Point Pressure (LPP) testing. For LPP measurement, the vertical tilt table/intravesical pressure clamp model was used. (*See, e.g.,* J. Lee et al., 2001, "New Functional Sphincter Formation After Allogenic Muscle Derived Stem Cell Injection into Denervated Rat Urethral Sphincter", *J. Urol. (Suppl.)*, 165:254, Abstract 1033, as provided to the Examiner with applicants' response of April 24, 2003). Prior to this testing, the spinal cord was transected at the T9-T10 level in order to eliminate reflex bladder activity in response to increasing intravesical pressures. This suprasacral spinal cord transection does not interfere with the spinal continence reflexes of the bladder neck and urethra. Rats were then mounted on a tilt table with the axis of rotation positioned for constant bladder height in relation to the pressure transducer. Intravesical pressure was clamped by connecting a saline reservoir to the bladder catheter via pressure tubing. The reservoir was mounted on a metered vertical pole for controlled height adjustment. Intravesical pressure was increased in 1-3 cm H_2O steps from zero upward until fluid was seen leaking from the urethral meatus. The pressure at which leakage occurred was defined as the LPP. The average of three consecutive LPP was taken as a data point for each animal.

Histological evaluation. Immediately following the LPP measurements, the rats were sacrificed, and the proximal urethra was removed. Each urethra was fixed with paraformaldehyde, cryoprotected, and embedded in OCT (Tissue Tek). Tissue sections were cut (6 μm), mounted, and air-dried. Four slides were made and analyzed: One slide was stained with hematoxylin and eosin (H/E); two other slides from each urethra were stained immunohistochemically with fast myosin heavy chain, which stains striated

muscle, and anti-protein gene product (PGP 9.5), which stains nerves; the fourth slide was stained for LacZ expression.

Statistical analysis. All LPP data are presented as means ± SEM, and p-values <0.05 are reported as significant. Overall comparisons between groups were performed using Prism statistical software (GraphPad Software, Inc., San Diego, CA). A nonparametric two-way ANOVA with Bonferroni inequality post-hoc analysis was performed to detect differences between experimental and time matched HBSS and control groups.

Results for the first set of experiments as described in ¶6 of the Chancellor Declaration

Cystometry. Cystometry in all groups of animals showed bladder contractions that were comparable in amplitude and duration. No difference in either the intercontraction interval or the maximal detrusor pressure during voiding was seen among the groups. These results suggest that electrocauterization did not impair bladder function.

Leak Point Pressure (LPP) testing. The mean LPP of the control rats determined at 2, 4, and 6 weeks after the sham operation were 49.8 ± 1.3 cm H_2O, 51.2 ± 1.5 cm H_2O, and 51.6 ± 2.0 cm H_2O, respectively. The mean LPP of the cauterized rats (no MDC injection) determined at 2, 4, and 6 weeks after HBSS injection were 17.2 ± 1.4 cm H_2O, 26.9 ± 1.9 cm H_2O, and 25.5 ± 1.3 cm H_2O, respectively. The mean LPP of the cauterized rats 2, 4, and 6 weeks after MDC injection were 38.2 ± 2.2 cm H_2O, 43.1 ± 2.6 cm H_2O, and 51.5 ± 0.9 cm H_2O, respectively. **Figure 2** summarizes these results. When compared to cauterized rats injected with HBSS, the increased LPP seen in each group injected with MDC were significantly higher ($p<0.001$ for each of the 3 groups). When compared to control rats, the LPP seen in the experimental groups 4

and 6 weeks after MDC injection were not statistically different. By 6 weeks, the LPP of the rats which had received MDC injection treatment had become normal.

Histological results. **Figures 3a and 3b** show H/E staining of the mid-urethra of animals 4 weeks after HBSS and MDC injection, respectively. The striated muscle layer is disrupted in the cauterized urethra from animals injected only with HBSS (**Figure 3a**), while it remains intact in the cauterized urethra of the animals injected with MDCs (**Figure 3b**). **Figure 3c** shows LacZ staining of the cauterized urethra from animals injected with MDC. Cells expressing β-galactosidase are seen within the urethral wall. These MDCs are integrated within the striated muscle layer. **Figures 4a and 4b** show fast myosin heavy chain staining within the cauterized mid-urethra of animals injected with HBSS and MDCs, respectively, 4 weeks earlier. In **Figure 4a**, the striated muscle layer injected only with HBSS is disrupted. By contrast, in **Figure 4b**, the striated muscle layer from animals, which had been injected with MDCs, is intact. PGP 9.5 staining shows the presence of more nerve formation in all 3 groups that were cauterized and injected with MDCs, compared with the cauterized groups injected with HBSS only. **Figure 4c** shows PGP 9.5 staining within the cauterized mid-urethra from animals that were injected with HBSS 4 weeks before; and **Figure 4d** shows the same staining within the cauterized mid-urethra from animals that had been injected with MDCs 4 weeks before. It is noted that the urethra from animals injected with MDCs reveal many more nerves that are stained compared with urethra from animals injected with HBSS. (Compare, e.g., **Figure 4d** with **Figure 4c**).

Figure legends for the first set of experiments as described in ¶6 of the Chancellor Declaration

The legends to the figures referred to above and presented for the first set of experiments are provided as follows:

Figures 1a-1c: *Characterization of rat MDCs.* **Figure 1a** shows bright-field microscopy of MDCs obtained by the plating/culturing method described in the Chancellor application, used at day 6 following initiation of the culture. (Reduced from x400). **Figure 1b** shows desmin staining of same MDCs of **Figure 1a**. (Reduced from x400). 65% of the cells stained positive for desmin. The arrows point to the same cells in both figures. **Figure 1c** shows dystrophin staining of MDCs from day 6 of the plating/culture technique, injected into the gastrocnemius muscle of an *mdx* mouse. (Reduced from x100). The arrow points to the large amount of dystrophin produced.

Figure 2: *Comparative effect of MDC injection on LPP between groups of animals described in the first set of experiments.* When compared to cauterized rats injected with HBSS and matched with respect to time, the increased LPP seen in each MDC injected group were significantly higher (* denotes $p<0.001$ for each of the 3 pairs of groups). When compared to control rats and matched with respect to time, the LPP seen in the groups of animals at 4 and 6 weeks after MDC injection were not statistically different ("N.S." denotes not significant; "C" denotes control; "H" denotes HBSS injected animals and "M" denotes MDC injected animals.

Figures 3a-3c: *Histology of the cauterized mid-urethra 4 weeks after HBSS or MDC injection.* **Figure 3a**: H/E staining of cauterized mid-urethra of animals injected with HBSS. (Reduced from x400). The arrow points to the disrupted striated muscle layer. **Figure 3b**: H/E staining of cauterized mid-urethra injected with MDC. (Reduced from x400). The arrow points to the intact striated muscle layer. **Figure 3c**: LacZ

staining of cauterized mid-urethra of animals injected with MDC. (Reduced from x400). The arrows point to MDCs expressing β-galactosidase, which are situated within the striated muscle layer of the mid-urethra.

Figures 4a-4d: *Differences in striated muscle layer and innervation of the cauterized mid-urethra.* **Figure 4a**: Fast myosin heavy chain staining of cauterized mid-urethra of animals at 4 weeks after HBSS injection. (Reduced from x400). The arrows point to the disrupted striated muscle layer. **Figure 4b**: Fast myosin heavy chain stain of cauterized mid-urethra of animals at 4 weeks after MDC injection. (Reduced from x400). The arrow points to the intact striated muscle layer. **Figure 4c**: PGP 9.5 staining of cauterized mid-urethra of animals at 4 weeks after HBSS injection. (Reduced from x400). The arrows point to only a few stained nerve fibers. **Figure 4d**: PGP 9.5 staining of cauterized mid-urethra of animals at 4 weeks after MDC injection. (Reduced from x400). Arrows point to many stained nerve fibers.

Figures 1a-1c for the first set of experiments as described in Appendix 1 of the Chancellor Declaration

Figure 1a:

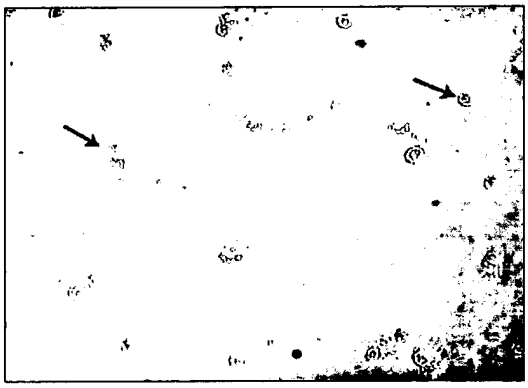


Figure 1b:

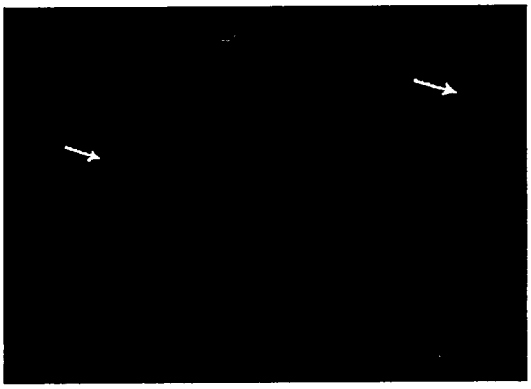


Figure 1c:

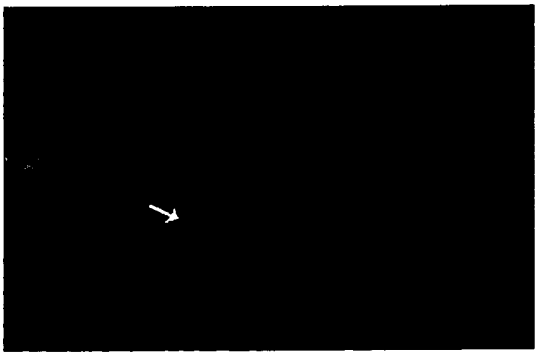
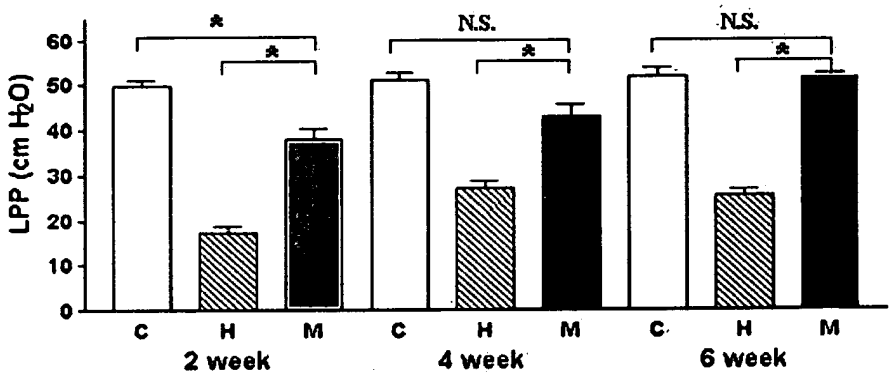


Figure 2 for the first set of experiments as described in Appendix 1 of the Chancellor Declaration

Figure 2:



Figures 3a-3c for the first set of experiments as described in Appendix 1 of the Chancellor Declaration

Figure 3a:

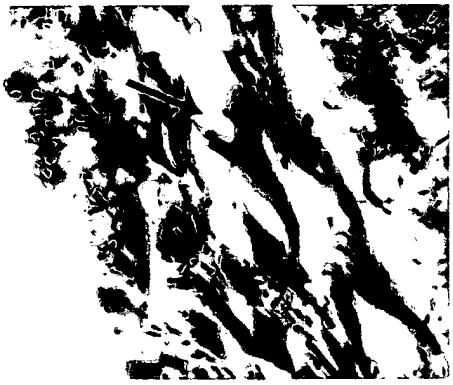


Figure 3b:

Figure 3c:

Figures 4a-4d for the first set of experiments as described in Appendix 1 of the Chancellor Declaration

Figure 4a:

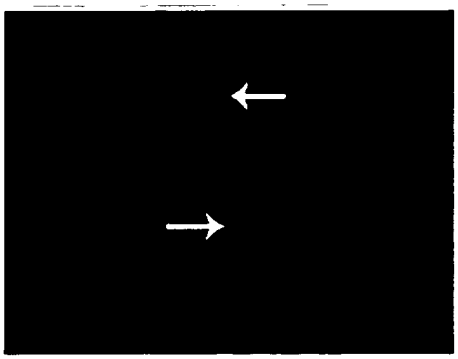


Figure 4b:

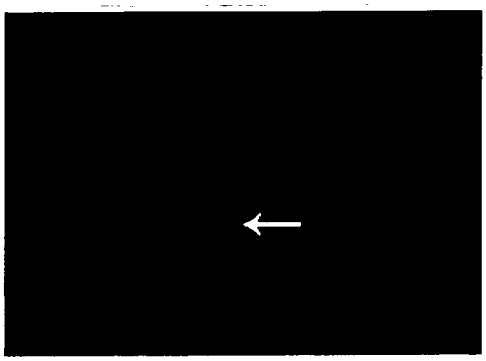


Figure 4c:

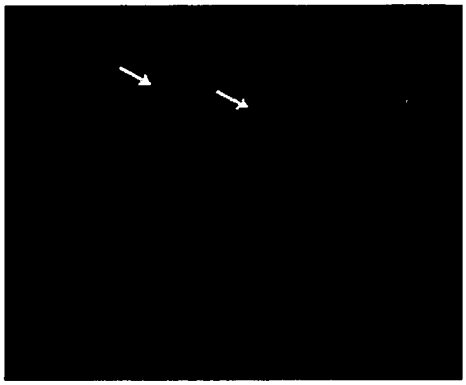
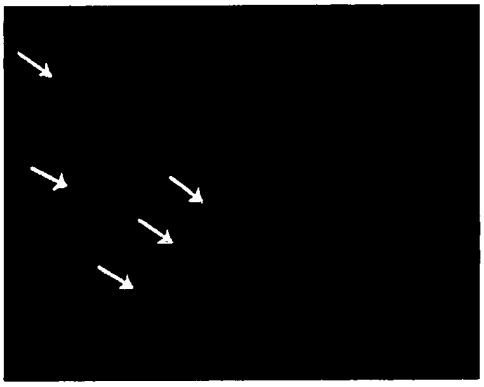


Figure 4d:



Appendix 2

Materials and methods for the second set of experiments as described in ¶7of the Chancellor Declaration

Animals and study design. As in the first set of experiments, normal female SD rats (6 weeks) were used in the second set of studies and experiments. Three experimental groups of female rats were established in this second study: (1) a sham-operated group of animals that received no injections (Control; C); (2) a urethral denervated group of animals that were injected with saline (D); and (3) a urethral denervated group of animals that were injected with MDCs (M). Each group underwent LPP physiology experiments at two time points: 1 week and 4 weeks post-surgery (n=5 at each time point for the C, D and M groups). Additionally, the bladders of 4 animals were injected with cells from either an "early plating time" in the method ((e.g., days 1-4; EP cells, (n=2)) or MDCs (n=2) to assess immunogenicity by monitoring the presence of activated CD8 lymphocytes 2 weeks post-injection.

Denervation of Sciatic Nerve: The sciatic nerve of the D and M groups of animals was denervated. The rats were given halothane anesthesia and after appropriate induction, bilateral dorsal incisions were performed over the ischiorectal fossa. Using an operating microscope, the sciatic nerve on each side was identified and transected distal to its origin from the vertebral column.

MDC Purification, Characterization and Injection: Muscle cells were harvested from the gastrocnemius of SD adult female rats and purified by the plating/culturing technique described in the Chancellor application. Briefly, a muscle biopsy was removed from the hind limb and minced into a coarse slurry using razor blades. The resulting muscle cells were enzymatically dissociated by adding collagenase-type XI (0.2%) for 1 hour at 37°C, dispase (grade II, 240 unit) for 30 minutes, and trypsin 0.1%

for 30 minutes. The muscle cells were then extracted and plated in a collagen-coated flask for 1 hour. All cells that did not adhere to the flask were then transferred to another flask for approximately 1 hour. Thereafter, the non-adhering cells were transferred to another flask and were incubated at 37°C overnight. This culture/transfer technique was carried out for an additional 4-5 days. Based on our previous studies using mouse cells and as described in the Chancellor application, the early plates (i.e., pp1-2) contain a majority of adhering fibroblasts while the late plates (pp5-6) are highly enriched for myogenic cells having the ability to differentiate into diverse types of muscle cells. The MDCs used in these experiments were taken from the plating at day 6 following initiation of the culture method. This population (called pp6) has been shown to express myogenic (desmin) markers, as well as stem cell markers (CD34). The proliferation medium used to grow the cells was Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum, 10% Horse Serum, and 1% Penicillin/Streptomycin.

Rats were anesthetized with halothane and a low midline incision was made to expose the bladder and urethra. A 10 µl Hamilton syringe was used to inject a total of 20 µl of MDC suspension in HBSS solution (3×10^6 cells per 20 µl). Two injections per rat (10 µl each) were made with microscopic guidance into either side of the urethra.

Leak Point Pressure (LPP) Measurement: At 1 and 4 weeks after MDC injection, LPP was measured using the vertical tilt/intravesical pressure clamp model of stress urinary incontinence. The animals were anesthetized with urethane (1.2 g/kg), and a transvesical catheter with a fire-flared tip (PE-90) was inserted in the dome of the bladder, and intravesical pressure was varied in 1-3 cm H_2O steps from zero upward until visual identification of leak point height. The pressure at leak point was taken as the Leak Point Pressure (LPP). The average of three consecutive LPP was taken as a data point for each animal in the vertical position (**Figure 5**).

Tissue Harvest and Histology: Immediately following the LPP measurement, the proximal urethra was removed. The tissues were then snap frozen using 2-methylbutane pre-cooled in liquid nitrogen. The area around each injection site was cryosectioned, Hematoxylin/Eosin stained, examined microscopically, and photographed.

Immunohistochemical staining for CD8-activated lymphocytes: Muscle tissue sections were fixed with cold acetone for 10 minutes and non-specific binding sites were blocked with goat serum (5%) in phosphate buffered saline. The sections were incubated with avidin D blocking solution for 20 minutes, rinsed briefly with PBS, and then incubated for 20 minutes with biotin blocking solution (4 drops per 1 ml of the diluted blocking serum per tissue section; Vector, CA). The sections were next incubated for 1 hour at room temperature in primary antibody (mouse monoclonal antibody against CD8 (Pharmingen, CA)). Sequentially, the endogenous peroxidase activity was blocked with 1% hydrogen peroxidase for 5 minutes, followed by several rinses in PBS. The sections were then incubated with Vectastatin Elite ABC (5 ml PBS plus two drops of Reagent A and Reagent B; Vector, CA) for 30 minutes. The peroxidase activity was determined using 3', 3'-diaminobenzidine (1 mg/ml; Sigma) and hydrogen peroxidase (0.03%). Hematoxylin was used for counterstaining. (**Figures 7A-7F**).

Statistical Analysis: LPP data are presented as mean ± S.E. Statistical analyses were performed using Student's t test for paired or unpaired data, where applicable. Comparisons between groups were performed using a one-way factorial analysis of variance, followed by Turkey post hoc test. A p-value of less than 0.05 was accepted as significant.

Results obtained from the second set of experiments as described in ¶7 of the Chancellor Declaration

Isolation of rat skeletal MDCs via the plating/culturing technique described in the Chancellor application: An MDC population of cells (pp6) was isolated by the preplate technique as described in the Chancellor application. The cells that took 5-6 days to adhere to collagen-coated flasks were round in nature, in contrast to the traditional morphology of myoblasts. The MDC population obtained and isolated from rat skeletal muscle had the characteristics of morphology, adherence, marker expression and survival post-injection as found using mouse skeletal muscle starting tissue.

Histological Analysis of Control (C), Denervated (D), and MDC-injected (M) Urethral Sphincter: The normal rat female urethral sphincter contains smooth and skeletal muscle. Hematoxylin and eosin (H/E) staining of the normal (control) urethral sphincter illustrates these muscle layers at low magnification (**Figure 6A**). The smooth muscle portion of the urethral sphincter consists of thick bundles of tightly packed smooth muscle cells (**Figure 6B**). In the denervated group of rats, the proximal urethral sphincter was atrophic at 4 weeks (**Figures 6C, 6D**). MDC injection into the denervated proximal urethral sphincter led to increased dorso-lateral skeletal muscle masses with variable fiber orientation at the injection sites (**Figures 6E, 6F**).

Immunohistochemical Staining For CD8 Lymphocytes: The early plating cells ("EP" cells, or "non-MDC") and the MDCs were injected into four rat bladders. Bladder tissue sections were stained for the presence of activated CD8 lymphocytes at 2 weeks post-injection. In the tissue injected with non-MDC, CD8 lymphocytes (red) were observed throughout the injection site (**Figures 7A, 7B**). In contrast, CD8 lymphocytes were not observed in the non-injected control (**Figures 7C, 7D**), or in the MDC-injected

bladder (**Figures 7E, 7F**), thus demonstrating that only the non-MDCs triggered an immune response following injection.

Leak Point Pressure (LPP) at 1 and 4 weeks: At 1 week, the LPP of the animals in groups C, D and M was 25.2 ± 1.9 cmH_20, 28.6 ± 0.8 cmH_20, and 36.7 ± 2.3 cmH_20, respectively. (**Figure 8A**). At 4 weeks, the LPP of animals in the groups C, D and M was 25.8 ± 2.5, 18.6 ± 5.2 and 44.1 ± 6.6 cmH_20 (**Figure 8B**). At 1 week after sciatic nerve transection, the LPP of animals in group D was not significantly different from that of group C. At 4 weeks after sciatic nerve transection, the LPP in the animals of group D was significantly lower than that of the animals in group M (p=.01). There was a significant difference between the LPP of animals in group C versus that of the animals in group M at both 1 and 4 weeks. There was a significant difference between the LPP of animals in group D versus that of the animals in group M at both 1 and 4 weeks (p=.001). There was no significant difference in LPP between 1 week and 4 weeks in group M.

Figure legends for the second set of experiments as described in Appendix 2 of the Chancellor Declaration

The legends to the figures referred to above and presented for the second set of experiments are provided as follows:

Figure 5: The three experimental groups, i.e., C, D, M, were examined for leak point pressure using the vertical tilt/ intravesical pressure clamp model of stress urinary incontinence.

Figures 6A-6F: H/E staining of rat female urethral sphincter revealed the normal anatomical structure of a sham-operated control (C group) animal (**Figures 6A, 6B**). Following denervation (D group), the circular skeletal fibers of a denervated proximal urethral sphincter were atrophic at 4 weeks (**Figures 6C, 6D**). MDCs injected into a denervated proximal urethral sphincter (M group) led to increased dorsolateral skeletal muscle masses with variable fiber orientation at the injection sites (**Figures 6E, 6F**). Magnification: A, C, E -- 10X; B, D, F -- 20X.

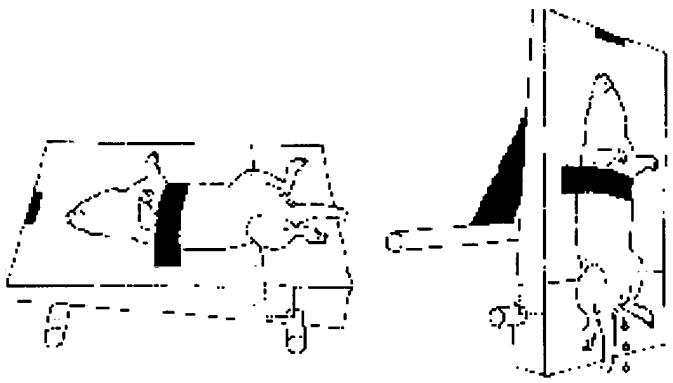
Figures 7A-7F: Immunohistochemical staining of bladder sections for CD8 activated lymphocytes revealed that only non-MDC cells triggered an immune response following injection into the bladder (**Figures 7A, 7B**). Like the non-injected control (**Figures 7C, 7D**), bladders injected with MDCs (**Figures 7E, 7F**) did not demonstrate any significant CD8 activity, thus suggesting that these cells do not trigger an immune reaction as observed for non-MDC following injection. Magnification: A, C, E --10X; B, D, F -- 20X.

Figures 8A and 8B: At 1 week following injection with MDCs, the LPP of the C, D and M groups of animals in the second set of experiments were 25.2 ± 1.9 cmH_20, 28.6 ± 0.8 cmH_20, and 36.7 ± 2.3 cmH_20, respectively (**Figure 8A**). At 4 weeks, the

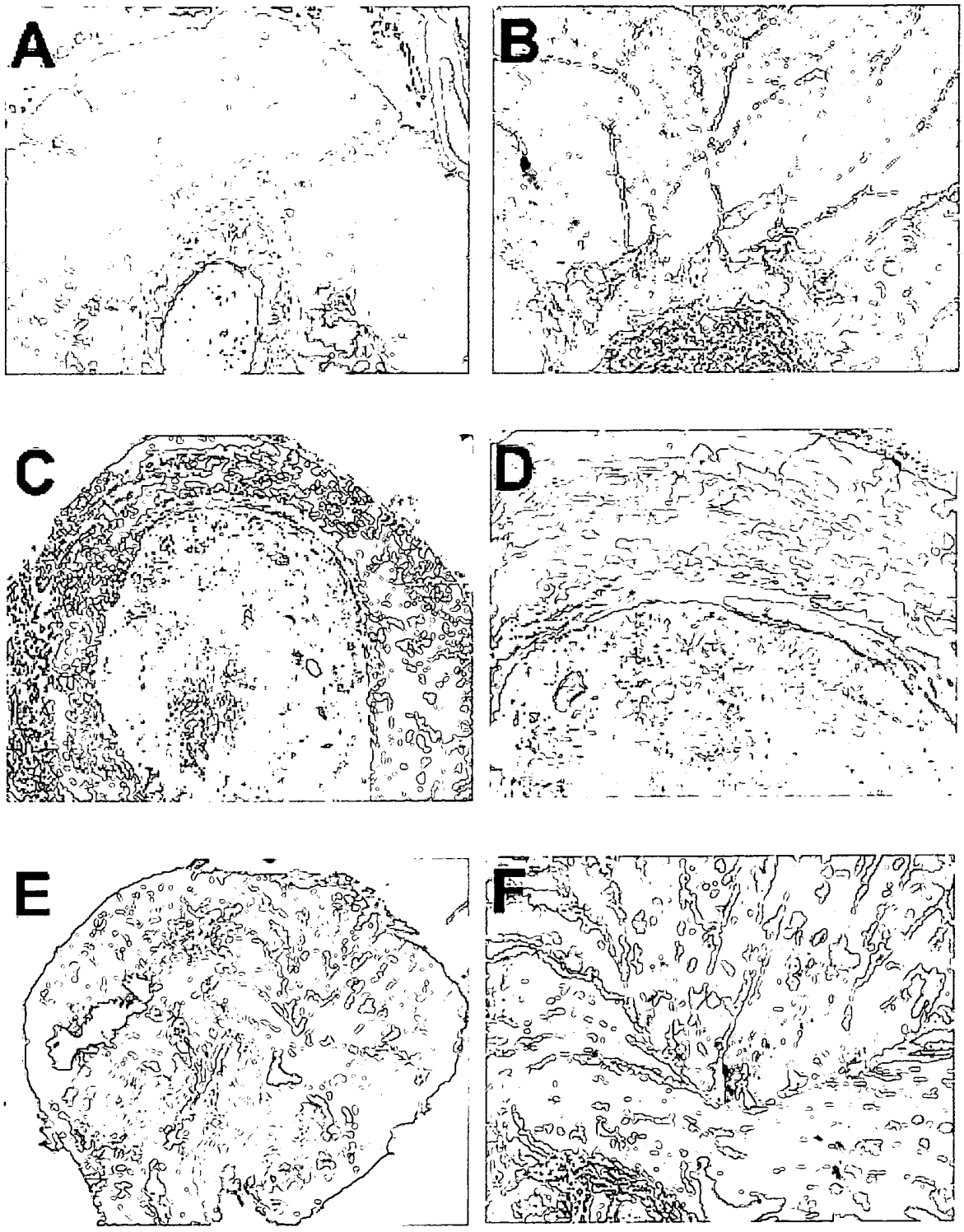
LPP of the C, D and M groups of animals in the second set of experiments were 25.8 ± 2.5, 18.6 ± 5.2 and 44.1 ± 6.6 cmH_20 (**Figure 8B**).

Figure 5 (page 20) for the second set of experiments as described in Appendix 2 of the Chancellor Declaration

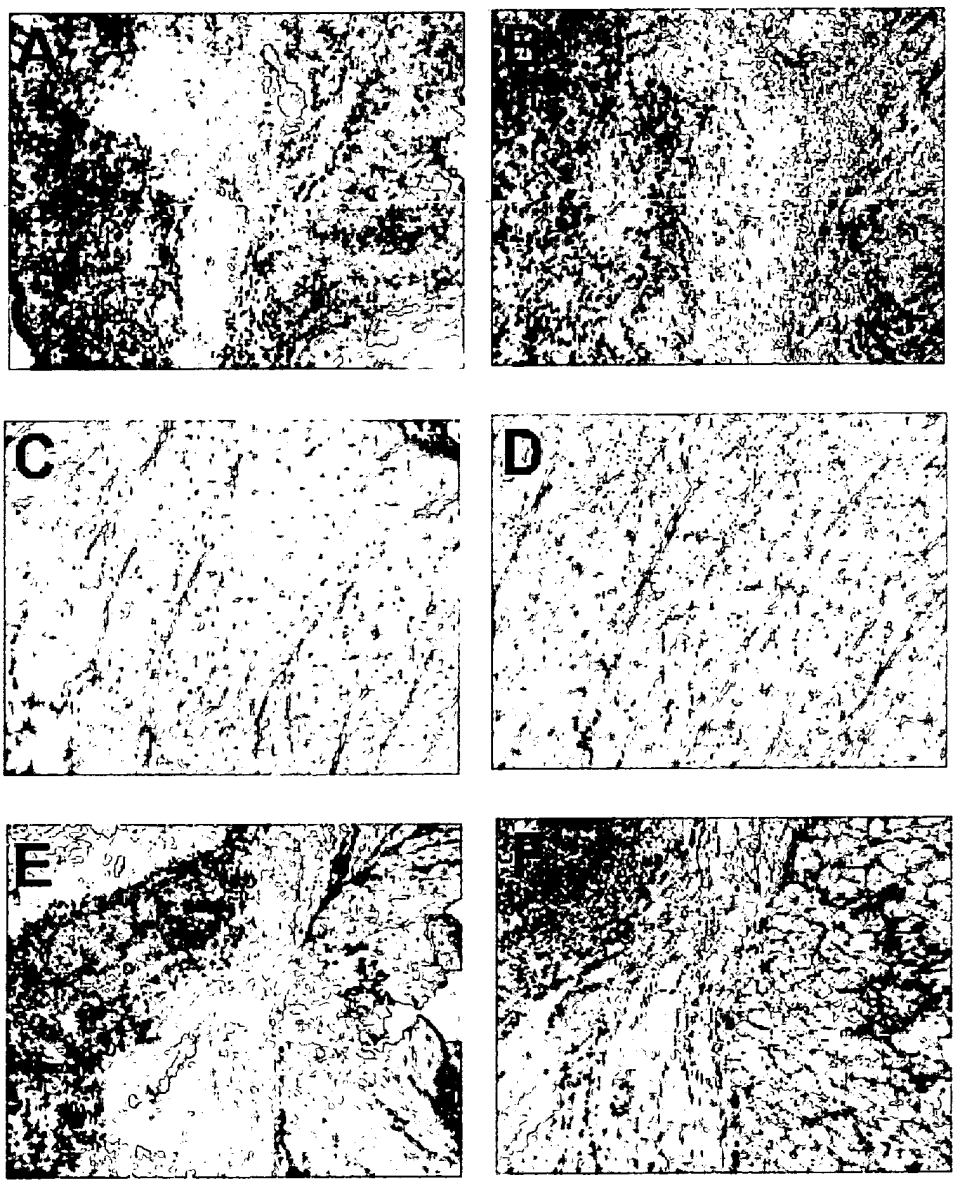
The Rat Continence Tilt Table



Figures 6A-6F (page 22) for the second set of experiments as described in Appendix 2 of the Chancellor Declaration


A
B
C
D
E
F

Figures 7A-7F (page 24) for the second set of experiments as described in Appendix 2 of the Chancellor Declaration


A
B
C
D
E
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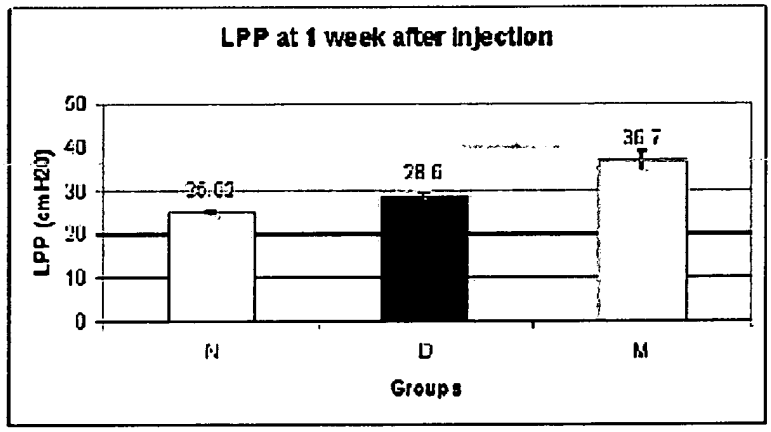
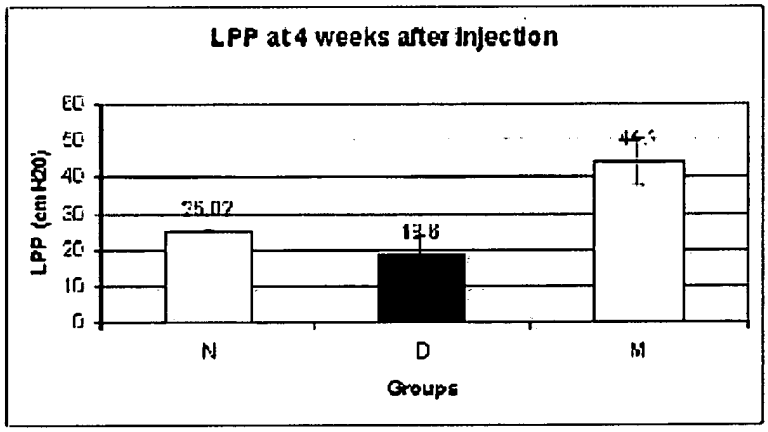
Figures 8A and 8B (page 26) from the second set of experiments as described in Appendix 2 of the Chancellor Declaration

A


LPP at 1 week after injection
LPP (cmH20)
50
40
30
20
10
0
25.02
28.6
36.7
N
D
M
Groups

B


LPP at 4 weeks after injection
LPP (cmH20)
60
50
40
30
20
10
0
25.02
13.6
N
D
M
Groups

This guidance was written prior to the February 27, 1997 implementation of FDA's Good Guidance Practices, GGP's. It does not create or confer rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. This guidance will be updated in the next revision to include the standard elements of GGP's.

DRAFT GUIDANCE FOR PRECLINICAL AND CLINICAL INVESTIGATIONS OF URETHRAL BULKING AGENTS USED IN THE TREATMENT OF URINARY INCONTINENCE

Urology and Lithotripsy Devices Branch
Division of Reproductive, Abdominal, Ear, Nose and Throat, and Radiological Devices
Office of Device Evaluation
Center for Devices and Radiological Health

November 29, 1995

TABLE OF CONTENTS

I. PREAMBLE

This guidance document is intended to identify the features of a clinical inves that the Food and Drug Administration (FDA) would find acceptable in support of investigational device exemptions (IDE) applications for urethral bulking agent (UBAs) used in the treatment of urinary incontinence (UI). Development of this guidance document is based on information submitted to FDA in previous applicat and the published literature on UBAs.

UBAs are injectable suspensions of biological (e.g., collagen) or synthetic (e. polytetrafluoroethylene (Polytef)) materials in a fluid medium that are intende injected into the urethral tissues to increase tissue bulk, thereby increasing urine flow. This guidance document focuses on the indication of UI due to intr sphincter deficiency (ISD). ISD is a condition where the urethral sphincter is coapt which can be corrected by increasing its bulk artificially. Although the of urethral bulking are less clear for other etiologies of UI, the information this guidance document can be applied to any type or cause of UI that the spons wishes to pursue. Urethral bulking agents are injected either periurethrally o transurethrally into the proximal submucosa of the urethra to achieve urethral coaptation or closure. At the time of this draft, Contigen Bard Collagen Impla only UBA approved by FDA for the treatment of UI due to ISD.

Injectable collagens intended for the treatment of dermal defects have been reg class III devices since 1981; and Polytef paste was approved as a new drug by F 1972 for the treatment of vocal cord dysfunction. Injectable Polytef is consi transitional device which currently requires regulation as a class III device.

This draft guidance is intended to identify the basic questions asked by sponso developing UBAs. It is not intended to replace interactions with FDA to addres

questions about a specific product; however, it does provide a framework for pr data to FDA which sponsors can use in developing devices of this type. FDA encourages comments on this draft guidance document and will also continue to consider scientifically valid alternatives to the preclinical and clinical requ stated within. All comments should be directed to the branch chief, Urology an Lithotripsy Devices Branch (ULDB), Office of Device Evaluation (ODE), Center fo Devices and Radiological Health (CDRH), 9200 Corporate Boulevard, Rockville, Maryland, 20850, (301) 594-2194. It is also recommended that the sponsor of a investigation contact ULDB prior to submission of an original IDE application.

II. DEVICE DESCRIPTION

The description of a urethral bulking agent shall include the names and amounts the materials/chemicals used (e.g., polytetrafluoroethylene, glutaraldehyde cro collagen), pH of the paste or suspension, and an explanation of how the device its intended function. In addition, if the materials have been used in other m applications, a description of these applications should be provided.

A. Synthetic materials

For devices utilizing synthetic materials, the description of their chemic structure should include the nature of any repeating groups, the nature of end groups, and the composition of possible branches and cross-links. (In spectroscopy and nuclear magnetic resonance spectroscopy are often used to identify chemical groups; if the polymers are insoluble, then attenuated t reflectance is another test to be considered.) If applicable, the molecul distribution (MWD), including number (Mn) and weight (Mw), average molecular weight, and polydispersity ratio (Mw/Mn) should also be provided

Physical/chemical characterization of the polymeric material, chemical characterization and quantification of the extracts of the polymer (e.g., and nonpolar solvent extracts), percentage of the polymer by weight (becau catalysts, fillers, etc. may be present), polymer particle shape and size percentage of particles by weight, other specifications (e.g., pH), and th method used to determine particle size should be provided to fully describ device.

If the device contains a chemically crosslinked polymer, information on th nature of the crosslinks and the degree and reproducibility of crosslinkin should be included. Determination of the amount of unreacted crosslinker measurement of equilibrium swelling of the polymeric component in a good solvent may provide this information.

This information should be supplied for all specific polymeric materials u manufacture the device and can be supplied as part of a submission to the or by reference to appropriate Drug and Device Master Files. If the devic manufacturer is buying polymer resins, the information should be available from the supplier.

B. Biological Materials

For devices utilizing biological materials, the animal source, tissue sour purity of the material, physical/chemical characterization of the critical material(s), analytical methods used for characterization including the ge nature of any chemical treatment or modification, and manufcturing specifications (acceptance/rejection criteria) should be included.

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For Collagen Containing Devices

The purity of the material should be demonstrated by gel electrophoresis photographs (original photographs, not photocopies). The gel photographs

should also document the types of collagen (type I, II, III, IV) present a quantify them. Differential scanning calorimetry data and enzymatic resis (to trypsin and chymotrypsin) data should be included to show that the dev material is mostly native collagen, not denatured collagen. In addition, glutaraldehyde cross-linked collagen, the degree of crosslinking and the aldehyde levels in the final product should be identified by appropriate s methods.

III. MANUFACTURING DATA

A. Methods, Facilities, Raw Materials, and Controls

Although sponsors are not required to adhere to current good manufacturing practices (CGMPs) for devices being studied under an IDE, the methods, facilities, raw materials, and controls used in the manufacture, processin packaging, and storage of the device should be provided in sufficient deta that a person generally familiar with CGMPs can make a knowledgeable judgement about the quality control used in the manufacture of the device. schematic presentation of the manufacturing process from the starting mate to the final product with identification of the critical steps should be p For synthetic polymeric materials, this description should include catalys curing agents, intermediate precursors, fillers, and colorants used. Wher possible, all materials should be identified by their chemical names.

Manufacturing guidance is available in the document entitled "Guidance for Preparation of PMA Manufacturing Information" available upon request from the Division of Small Manufacturers Assistance (DSMA), HFZ-220, CDRH, FDA, 1350 Piccard Drive, Rockville, Maryland 20850.

B. Sterilization

Sterilization information should include standard operating procedures for qualifying the sterilization process and sterilizing the device. Since th a permanent implant, adequate information on the method of sterilization, sterilization validation protocol/results, sterility assurance level, and description and validation protocol/results should be provided to demonstr device sterility. For ethylene oxide (EO) sterilization, residual levels ethylene glycol, and ethylene chlorohydrin remaining on the device (after sterilization and quarantine period) should be included. If radiation ste is used, the maximum and minimum radiation doses should be specified.

Although not required for IDE approval, a description of the protocol for life studies should be provided, with justification of the sample size and results of the study. Shelf life should be based on the ability of both t and the package to maintain their integrity. Studies should include the e temperature, humidity, pressure and light exposure, as well as shipping an handling (dropping and vibration). After subjecting the packaged devices simulated or real-time environment, the devices should be tested for steri material integrity. (Shelf-life studies are sometimes overlooked when pla a clinical study resulting in a marketing application which may unnecessar limit the shelf-life of the device. Submission of the shelf-life protocol IDE will enable FDA to comment on the adequacy of the protocol which may avoid unnecessary labeling restrictions. It may also benefit the sponsor may serve as a reminder to set aside samples for real-time storage.)

C. Pyrogen Testing

Pyrogen/endotoxin testing protocols and results should be provided to docu the non-pyrogenicity of the device, including bioburden data, and the endo detection limits of the tests. Endotoxin levels in the device should be determined by the Limulus Amebocyte Lysate (LAL) test (see USP) due to its greater sensitivity than pyrogen testing in rabbits (USP).

IV. DEVICE ACCESSORIES

A brief description of the periurethral and/or transurethral accessories (e.g., needles, catheters, etc.) proposed for use in the clinical investigation should provided. This information should include the name of the supplier of each acc (if applicable), sterilization data, and previous regulatory status of the acce new device or a previously cleared device for urological use (including the 510 number, if known)).

V. PRECLINICAL DATA

The biocompatibility/animal data required depends on the specific urethral bulk agent selected and whether it is a biological material or a synthetic polymeric However, regardless of the nature of the material, the animal studies should re worst case scenario and should be conducted on samples of the final sterilized (i.e., the product that requires no further processing for clinical use). Furt order to maximize the relevance of the long-term implantation studies, the test should be conducted in large animals (e.g., dogs) simulating the clinical use c regarding the implant site (urethral mucosa) and volume of the implant to the e possible.

A statement should be provided that all non-clinical studies have been conducte accordance with the Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies regulation (21 CFR, Part 58). All deviations from the GLP regulation s be described fully, including a justification for accepting the results of thes any study was not conducted in accordance with GLPs, a statement of the reason noncompliance should be included.

In order that an independent evaluation of the study conclusions can be made, c reports including detailed test protocols, study results, study conclusions, an on all adverse events should be provided for all studies. For those studies re histological examination, results from serial sectioning and staining (preferab evaluated by a blinded, independent pathologist) should be provided. These stu should include actual representative photographs of the microscopic histology w possible (due to limited reproduction capabilities of photocopies).

A. Synthetic Materials

Synthetic polymeric materials should be tested to demonstrate that they ar toxic upon long-term intimate contact with the body. Even high molecular weight polymeric materials contain low molecular weight components, such a monomers, oligomers, and catalysts which can leach out into the body. Therefore, one important requirement of the preclinical toxicology testing device is to determine the potential toxicity of the previously identified releasable chemicals (section II.A) as they appear in the final sterilized These biocompatibility tests should reveal the potential for local as well systemic toxicity (including genotoxicity, carcinogenicity, adverse reprod effects, teratogenicity, and immunotoxicity) of any leachable substance. when appropriate, the chemicals recovered by extraction of the final steri device can be used as the test article in animal studies.

In addition, a significant concern for any implanted device is its potenti cause cancer. This potential may arise not only from chemical leachables degradation products from the device, but also from physical effects of th device at the implanted site. Therefore, if data are not available in the to address this issue, then adequate long-term studies with implantation o device materials should be conducted to evaluate the carcinogenic potentia the device. If adequate justification (or data from previous submissions FDA) is provided, these tests do not need to be completed to obtain IDE approval.

The biocompatibility testing required for synthetic materials should be conducted in accordance with Blue Book memorandum # G95-1 entitled "Use of

International Standard ISO-10993, Biological Evaluation of Medical Devices Part 1: Evaluation and Testing" (obtainable through DSMA), which includes FDA-modified matrix that designates the type of testing needed for various medical devices. For a synthetic UBA, the applicable tests are those indi for permanent implants contacting mucosal tissue. These tests include:

- intracutaneous irritation test,
- acute systemic toxicity test,
- cytotoxicity,
- dermal sensitization test (Magnusson-Kligman test),
- hemolysis,
- muscle implantation test,
- mutagenicity (genotoxicity),
- pharmacokinetic/biodegradation studies,
- subchronic toxicity,
- chronic toxicity,
- reproductive and developmental toxicity, and
- carcinogenesis bioassay.

Some of the studies cited above (e.g., pharmacokinetics, reproductive and developmental toxicity, and carcinogenesis) are required only for material are suspected of causing serious adverse effects.

While intracutaneous irritation, acute systemic toxicity, cytotoxicity, he and muscle implantation testing provide information on the short-term toxi of the device extracts (muscle implantation test is an exception) in anima long-term studies provide data on the implant site tissue reaction over ex periods of time. It is not necessary that all of the long-term studies be completed prior to submission of the IDE.

For the above testing, if certain components in the device are known to be materials (e.g., saline, glycerin), it is sufficient to only test the extr polymer component. However, both polar and non-polar solvent extracts should be tested. The purpose of using non-polar solvents is to determine leachability of the small molecular weight, hydrophobic components from th polymer matrix. This rationale should govern the choice of the non-polar solvent.

Mutagenicity testing should, at a minimum, consist of bacterial mutagenici mammalian mutagenicity, DNA damage, and cell transformation assays.

Of special concern in the pharmacokinetic/biodegradation studies of the im are questions relating to the ultimate fate, quantities, sites/organs of d and routes of excretion. The clinical significance of particle migration also be addressed.

Acute, subchronic and chronic toxicity, carcinogenicity*, reproductive and teratological effects*, and immunotoxicity* studies should be conducted on final sterilized device. If the whole device cannot be used, the device m or extracts of the device can be used. Dose response and time to response should be characterized. Complete reports from acute, subchronic, and chr toxicity testing of the final sterilized device, the device components, or device extracts should include gross and histopathological studies in appr tissues both surrounding and remote from the implanted site. For more spe guidance on these tests, please contact ULDB at (301) 594-2194.

Tissue Reaction Study: Gross histology and microscopic histology of impla sites at different times (1 week and 1, 3, 6, 12, and 24 months) are consi necessary for evaluation of short-term and long-term tissue reactions. Th study should also provide information on whether the implanted material is retained or absorbed over time.

Particle Migration Study: This study involves examination of nearby

lymphatics and distant organs, such as lungs, liver, kidneys, etc., for mi of particles of the implanted material. If the particles are not radiopaq serious consideration should be given to labeling the particles with a rad isotope to facilitate detection of the particulate material. It should be this study could be part of the long-term tissue reaction study.

Immunotoxicity studies: If a urethral bulking agent contains materials wh have potential to produce immune responses, at a minimum, the adjuvant eff of these materials and antibody production (including antinuclear autoanti should be studied. Examination of the thymus, spleen and regional (pelvic lymph nodes at 12 and 24 months in the tissue reaction study should provid clues regarding the immune response to the implanted materials.

B. Biological Materials

Many of the tests described above for synthetic polymeric materials are no necessary if the UBA is composed of biological materials. For instance, U intracutaneous irritation, acute systemic toxicity, cytotoxicity, hemolysi muscle implantation, hemolysis, mutagenicity, genotoxicity, and carcinogen tests may not be necessary. Particle migration studies to distant sites/o may not be relevant since biological materials tend to adhere to the impla and are degraded by enzymes. However, studies designed to assess the impl site tissue reaction which are described above for synthetic materials do and should be conducted. In addition, the following concerns are associat with biological materials.

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Bioabsorption: While synthetic materials are not generally absorbable, biological materials are degraded at the implant site by enzymes. The rat degradation depends upon the nature of the material and whether it is crosslinked. The amount of the implanted material remaining at the inject site typically decreases with time; after 6 to 12 months very little mater be found at the injection site. Therefore, bioabsorption (or biodegradati significant concern. A consequence of this biodegradation is that the pat for whom this implant is intended require reinjections periodically in ord maintain the improvement in urinary incontinence initially achieved. Ther it is important to determine how much (or what percent) of the implanted material remains at 3, 6, 12, and 24 months post implantation. Bioabsorpt can be assessed from the same studies designed to evaluate the long-term t reaction at the implant site, or from the injection of the radiolabeled bi material and determination of the radioactivity remaining at the injected different time periods after the injection.

Immunogenicity: Biological materials have the potential for causing an immunological reaction. For instance, a collagen implant is known to prod anticollagen antibodies. Therefore, humoral and cellular immunity to the biological material (i.e., the injectable implant) should be evaluated in choosing a species that is generally known to produce an immune response. Cellular immunity may be assessed by injecting the material intradermally assessing the delayed hypersensitivity reaction. Humoral response may be assessed by determining the antibody levels in the serum as a function of after injecting (implanting) the material at 3 weeks, 6 weeks, 3 months an months. The same study that is designed for evaluation of tissue reaction bioabsorption can be used for evaluating the humoral immune response. Examination of the thymus, spleen and regional (pelvic) lymph nodes at 12 24 months in the tissue reaction study should provide clues about the immu response to the implanted materials.

VI. CLINICAL STUDY DESIGN

The success of a clinical study is based on the overall coordination of three f the design of the study; the conduct of the study; and the analysis of the stud The sponsor should carefully consider and execute each step of the study accord

the initial overall study plan (statistics should be taken into account in both design and the study analysis). The clinical information collected should prov reasonable assurance of the safety and effectiveness of the device in the treat urinary incontinence due to intrinsic sphincteric deficiency and should constit scientific evidence as defined in 21 CFR 860.7(c)(2). All clinical study proto should include:

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a clear statement of the study objective(s) - The objective of the study should focused, clearly stated, and consistent with the research question(s) to be ans with the intended labeling claims for the device.

protocol development implementing the study design - When developing the protocol, the following study design issues should be considered: patients to b comparability of treatment groups with a control, selection of clinically relev outcome variables, and procedures to control potential sources of bias.

sample size determination - The number of patients to be enrolled should take account the number of patients needed to complete the study based on statistica calculations.

patient recruitment procedures - Patients should be enrolled in a manner which minimizes selection bias. The protocol should detail the procedure by which consecutive patients meeting the inclusion criteria are selected. All situatio a patient meets the inclusion/exclusion criteria but is not offered enrollment investigator (or the patient declines enrollment) should be documented.

baseline and follow-up assessments - These assessments should be clearly and concisely defined and should be measured by objective and standardized methods are detailed in the clinical protocol.

outcome variables or endpoints - Outcome variables should be objective, concise defined, and clinically informative about the condition and device being studie Blinded techniques for assessing these variables are preferred.

definitions of success and failure - So that valid conclusions can be drawn fro study, standard definitions of success, failure, and complications should be es prior to initiating the study.

The following sections provide specific details of a clinical study that FDA be essential in evaluating the safety and effectiveness of the device under invest

Pilot Study

FDA recommends that studies for urethral bulking agents used in the treatment o be conducted in phases to minimize the risks to investigational subjects and to clinical experience in using these devices prior to initiating large scale clin During the pilot study, the investigator(s) can gain valuable information regar safety, the injection techniques, appropriate sites for injection, volume of th be injected for urethral coaptation, and the need for reinjections. This study consist of one institution and 20 subjects, not including a control group. Alt recommended that the pilot study be randomized so that these patients can be po with the larger clinical study patients, this is not required since the primary this study is to establish reasonable safety and preliminary effectiveness prio a larger patient population with the experimental device. The study should mon adverse events.

Clinical Study

For expansion, a progress report on the first 10 subjects treated with at least follow-up (from the previous treatment date) should be submitted to FDA for rev and approval. This larger clinical study should consist of the following.

A. Control Population

A randomized, multicenter, masked (blinded) controlled study should be conducted to evaluate the safety and effectiveness of the device for the i use population. The use of a concurrent, masked control arm consisting of patients randomized to treat urinary incontinence and undergoing identical evaluation as the experimental group is strongly encouraged in order to ev the safety and effectiveness of the experimental treatment. FDA also recommends that randomization be blocked by site.

Currently, there is only one UBA approved by FDA (i.e., Contigen) to treat due to ISD; therefore, this would be the most appropriate choice for the c group. If chosen as the control, Contigen should be used in strict accord with its labeling. Other controls (e.g., using the patient as his/her own control) have also been suggested and may be appropriate, however these ty of studies typically do not address potential biases which may compromise overall data analysis.

The sizes of the treatment and control populations should be based on the expected probability of success for the two groups. The sponsor should determine the sample size needed to achieve a pre-defined significance lev with sufficient power to detect a pre-determined minimal difference which clinically meaningful for each of the hypotheses to be tested. (This prec FDA from being able to identify the number of patients needed for submissi of a future marketing application.) The minimum sample size should be the largest obtained from the sample size calculations for testing each of the hypotheses in question so that a few patient losses will have less chance invalidating the study.

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B. Patient Selection Criteria

The following should be considered when identifying the intended patient population:

1. Inclusion Criteria

- patient has UI due to ISD,
- patient is at least 18 years of age,
- patient's incontinence has not shown any improvement for at least 12 months,
- patient has failed prior noninvasive treatments (e.g., behavior modification, bladder exercises, biofeedback, electrical stimulation, and/or drug therapy),
- patient has good bladder function,
- patient has viable mucosal lining at the likely sites of injection (e bladder neck),
- patient has a negative urine culture,
- patient agrees to sign the informed consent document,
- patient is mentally competent and able to understand all study requirements,
- patient has a life expectancy of at least 1 year,

- patient agrees to be available for the follow-up evaluations as requi by the protocol,

2. Exclusion Criteria

- patient has vesicoureteral reflux, spastic bladder, detrusor instabil high pressure instability,
- patient is on current medication for UI,
- patient has UI of neurogenic etiology,
- patient used indwelling catheters for a long period of time and has fibrosis of the tissue at the likely injection sites,
- patient has received pelvic radiotherapy and has fibrosis of the tiss the likely injection sites,
- patient is pregnant, lactating, or planning to become pregnant in the 12 months,
- patient has any condition which could lead to significant postoperati complications, including current infection, uncontrolled diabetes, or elevated residual urine from bladder outlet obstruction,
- patient is morbidly obese (defined as 100 pounds over their ideal bod weight according to Metropolitan Life Insurance Co. tables) and would not be expected to benefit from treatment,
- patient has current or acute conditions involving cystitis or urethri
- patient has any condition that would preclude treatment due to contraindications and/or warnings in the experimental or control prod labeling, and
- if applicable, patient is allergic to any bovine collagen product or positive reaction in the Contigen skin test or is undergoing or inten undergo desensitization injections to meat products.

C. Preliminary Screening for Enrollment

The following information should be collected through a 2-week diary, medi history, and a validated questionnaire for preliminary screening of subjec determine whether they have UI, likely due to ISD. At a minimum, the diar and medical history should include the following:

2-Week Diary

- involuntary urine leakage,
- frequency of urine leakage,
- number of pads used,
- urine leakage during sleep,
- urine leakage during stressful activity, coughing, sneezing,
- urine leakage while sitting or standing,
- presence or absence of urgency to empty the bladder,

Medical History

- prior surgeries (including dates) for problems other than UI,
- prior surgeries (including dates) for UI,
- prior drug therapy (including dates) for UI,
- prior pelvic floor exercise therapy, biofeedback, and/or

electrostimulation therapy (including dates) for UI,
- duration of UI with no improvement at the time of the visit,
- present or past bladder or kidney infections (frequency and dates),
- current medications,
- current management of condition,
- history of allergies,
- connective tissue (autoimmune) diseases, and
- current or past malignancy.

D. Pre-treatment Evaluation

Pre-treatment (and post-treatment) tests should be clearly defined and con methods should be used at all investigational sites. The pre-treatment ur evaluation should rule out by appropriate differential diagnostic measures significant coexisting disease/condition that might confound the study dat analysis.

The patients who are found to have UI, likely due to ISD, in this prelimin screening should undergo the following evaluations to confirm the diagnosi UI due to ISD and to exclude cystocele, enterocele, uterine prolapse, uret hypermobility, or neurologic disorder as contributing factors to UI:

1. a complete history and physical examination;

2. uroflowmetry: voided volume (with a prospectively defined minimum to ensure meaningful analysis, e.g., 125 ml), total time of voiding, pea flow rate, average flow rate, and post-void residual volume (measured by ultrasound or catheterization, but consistent methods should be us pre- and post-treatment);

3. cystometry;

4. valsalva leak point pressure (LPP) - (LPP is the intravesical pressur which urine leaks around the catheter through the urethra during urodynamic evaluation. This is determined by inserting a catheter in the bladder and filling it with 200-250 ml of water (see Rodney Appel World Journal of Urology (1990), 8:208-211; Gopal Baldani et al., Contemporary Urology, July 1993, pp. 29-35; Edward McGuire et al., Journal of Urology (1993) 150:1452-1454). LPP is usually low (below 50-60 cm of water) for ISD patients. The clinical protocol should include a description of the procedure used for LPP determinations);

5. urinary incontinence scale - The incontinence grading scale of 0 to 3 described by Stamey in Campbell's Urology is acceptable to FDA. (Refer to section G of this guidance for more information);

6. pad weight test;

7. urinalysis and urine cultures (to rule out urinary tract infection (U

8. pulmonary and liver function, and blood chemistry including CBC, BUN, and creatinine clearance (if the UBA is a biological material or potentially immunogenic synthetic polymeric material, the production antibodies to the injected UBA should be monitored);

9. cystoscopic examination to document the absence of bladder neck obstruction, presence/absence of urethral strictures, bladder patholo

10. quality of life assessment, including sexual function/dysfunction; an

11. pregnancy test, if applicable.

Based on the above evaluation, selected patients should not have detrusor

instability; they should have adequate bladder capacity (350-500 ml) and nor post void residual urine (less than 50 ml). A patient with a UTI should be t this condition with antibiotic therapy prior to receiving the control or exp treatment. For a clinical study in which the control group patients receive it is mandatory that those selected for treatment (Contigen or experimental pass the collagen skin test described in Contigen labeling. According to thi 0.1 cc of Zyderm collagen should be injected intradermally into the volar fo If erythema or edema appears at the injection site during the next 30 days, is considered to have a positive reaction and becomes ineligible for partici the study. This skin test identifies the subjects with preexisting hypersens bovine dermal collagen and excludes them from the study. If randomization ta prior to the skin testing, then a placebo skin test could be performed for t experimental treatment if it does not contain collagen. If however, the expe treatment contains another immunologic material, appropriate skin testing fo material should be developed and utilized.

E. Post-Treatment Evaluations

Post-treatment evaluation should be conducted at 1, 3, 6, and 12 months, and intervals thereafter until marketing approval. Longer term follow-up may be (pre and/or post approval) depending on the properties of the device materia need for reinjections, and/or the ability to provide adequate information re safety and effectiveness of the device. Intraoperative and post-treatment ad should be completely detailed. Post-treatment evaluation at each visit shoul conducted in the same manner as the pre-treatment evaluation and, unless oth specified, should include:

1. 2-week patient diary;
2. physical examination;
3. uroflowmetry: (to be consistent with pre-treatment evaluation);
4. KUB immediately following injection and at 6 and 12 months post- treatmen document any migration of particulate material;
5. cystometry on all patients at 6 and 12 months post-treatment (Urodynamic results regarding detrusor stability, bladder volume, first voiding sensatio should be recorded);
6. valsalva leak point pressure at 6 and 12 months post-treatment;
7. urinary incontinence scale;
8. pad weight test;
9. urinalysis and urine cultures;
10. pulmonary and liver function, and blood chemistry including CBC, BUN, an clearance (if the UBA is a biological material or a potentially immunogenic polymeric material, the production of antibodies to the injected UBA should 11. cystoscopic examination at 6 and 12 months post-treatment; and 12. quali assessment at 6 and 12 months and yearly thereafter, including sexual functi dysfunction. Quality of life assessments are not mandatory for IDE approval considered helpful for determining efficacy in a future marketing applicatio experimental or control agent are suspected to be immunogenic, the patients advised to report any problems indicative of connective tissue disease (e.g. ache, joint pain, skin rash) to the investigator; upon receipt of these comp the investigator should determine whether the patient should be referred to rheumatologist. If, in the opinion of the rheumatologist, there is any likel the treatment may have contributed to the adverse effect, the patient should further UBA injections.

F. Injection Procedures

The protocol should contain a brief description of the equipment (e.g., need catheters, cystoscopes) and procedures (periurethral, transurethral) that wi for injecting the UBA. This description should also include the preparation use of antibiotics and anaesthesia, and the site(s) of injection.

G. Urinary Incontinence

Scale The severity of the patient's UI should be graded by the investigator information provided in the patient's diary, the patient's responses to the and from the pre- and post-treatment evaluations. The incontinence grading s 3 described by Stamey in Campbell's Urology (Fourth Edition, W.B. Saunders C pp. 2272-2293) is acceptable to FDA. The assignments according to this scale Grade 0 : continent (dry); Grade 1 : urine leakage is associated with stress activities, such lifting weights, coughing, or sneezing but never in bed at Grade 2 : urine leakage is associated with activities of minimal stress, suc or standing up; Grade 3 : urine leakage occurs at all times, with any activi irrespective of position.

H. Data Forms

Appropriate data forms for screening, pre-treatment, treatment (procedure re and post-treatment evaluations, including the 2-week patient diary format an questionnaires, should be included with the clinical protocol. The screening pre-treatment forms should provide for assessment and documentation of patie previous therapies/surgeries, physical examination findings, urodynamic and for the differential diagnosis of UI due to ISD,patient diary observations t the baseline and follow-up incontinence grade, pulmonary and liver function urinalysis, urine culture, and pregnancy test. These data forms should also information regarding the result of the Contigen skin test (if applicable), rheumatological status of the patient (i.e., whether the patient has any con tissue/autoimmune disease). In general, the inclusion and exclusion criteria included in these data forms. The treatment forms should provide for documen patient identification (ID), physician name, treatment date, the type of ane (general, spinal or local), antibiotics used, the duration of the same or ot antibiotics to be used after the procedure, the injection procedure (periure or transurethral), assigned treatment (experimental versus control), the lot the UBA used, volume of the UBA injected, and any complications/ adverse eff observed or reported by the patient. The post-treatment (follow-up) forms sh for documentation of: patient ID, physician name, follow-up visit informatio (e.g., date of visit, follow-up interval), patient incontinence information patient diary to assess the incontinence grade, and the volume of the UBA in if the patient is retreated. Urodynamic testing results regarding detrusor s bladder volume, first voiding sensation, etc. should be recorded in the 6 an month visits. Pulmonary and liver function results, CBC, blood chemistry dat cystoscopy, and LPP test results should also be documented at the 6 and 12 m Additional post-treatment forms may contain information regarding adverse effects/complications and immune response (e.g., antibodies to the injected effects.

I. Analysis Considerations Safety

All adverse events, systemic or local should be monitored and documented. These include anaphylactic reactions, fever, symptoms of autoimmune/ connect disorders (e.g., joint pain, muscle aches), pain (perineal or injection site bleeding, leakage of the implant from the injected site (extravasation), inj injury, UTIs, fever, hematuria, abscess at injection site, urinary retention outlet obstruction. In addition, at appropriate intervals during follow-up, function, liver function, CBC, blood chemistries, and antibodies to the inje (if a biological or potentially immunogenic material is involved) should be comparison with pre-treatment values. Urine culture and urinalysis will prov insight into any underlying or developing urological problem. Effectiveness effectiveness of the UBA should be based on the urinary incontinence scale d earlier. This means that a decrease from the baseline (pre-treatment) incont (e.g., Grade 3 to 2, Grade 2 to 1, Grade 3 to 1, Grade 1 to 0, etc.), indica improvement, serves as a primary criterion for effectiveness. Clearly, a cha grades indicates more improvement than 1 grade. Any change to Grade 0 (dryne maximum improvement to be expected and is the gold standard for measuring th effectiveness of the device. To ensure uniformity between investigators, cri should be prospectively established to guide how these scores should be assi The evaluation of grade changes should be based on the information documente

patient diary (2-week diary) and the questioning of the patient by the inves at the follow-up visits. A conservative approach should be used in assigning changes. For instance, if a patient noticed leakage while walking or standin couple of occasions, but improved to Grade 1 most of the time, the conservat requires that the incontinence be assigned a Grade 2. Often after the treatm incontinence improves transiently; if the improvement does not last, it is n meaningful for the patient. Therefore, device effectiveness should be judged by the duration and magnitude of improvement (or dryness), not by transient The primary criterion of assessing incontinence grade changes is subjective. objective measure such as a significant increase in LPP would serve as a sec criterion for the device effectiveness. A patient often seeks treatment for quality of life considerations. It is also well reported in the literature t improvement in UI enhances the patient's quality of life. Due to this relati the fact that each patient perceives his/her incontinence differently, quali assessments are considered an important component in evaluating device effec

Patient Withdrawal

There should be a complete accounting of all patients in the study including for patient withdrawals/discontinuations (loss to follow-up should not excee the course of the study). In the event of death, every effort should be made autopsy reports.

Clinical Utility

The clinical data should be reported and analyzed to permit a determination utility (i.e., that the intervention provides clinically meaningful results patients). Labeling claims will be restricted to patient populations in whom utility (benefit) has been demonstrated. The issue of clinical utility is di further in Bluebook Memorandum P91-1 "Clinical Utility and Premarket Approva obtainable from DSMA.

Risk/Benefit

The clinical benefit of the UBA for the treatment of UI due to ISD will be e balancing the adverse effects versus the effectiveness results. This clinica will then be compared with that of the control. As stated earlier, the durat improvement is likely of greater significance than transient improvement. Th of retreatments (i.e., durability of the injectable agent) should also be as Achievement of dryness (continence) is the gold standard for measuring effec All adverse events should be reported and stratified to identify patients th potentially have a higher risk of complications and which demographic or oth parameters lead to less probability of effectiveness. A complete description adverse event should be presented which discusses its severity, duration and

Statistical

The statistical report should:

(a) compare all treatment data to the control;
(b) include statistical measures;
(c) stratify the safety and effectiveness data by gender, by the degree of i (decrease of 1, 2 or 3 grades, including dryness), number of treatments, vol the UBA injected, and by relevant pre-treatment patient characteristics (e.g., baseline incontinence grade, previous surgery),
(d) account for all patients at each follow-up period,
(e) provide summary tables for all important parameters (e.g., for improveme dryness, for adverse effects, for antibodies),
(f) provide justification for pooling results across investigational sites a discussion of any unusual results at any of the sites, and
(g) provide life table analyses presented separately for male and female pat